RJC

RASĀYAN *J. Chem.*
Vol. 12 | No. 1 |355 - 359| January - March | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

DETERMINATION OF ETHYL 2-CHLOROACETATE AS A GENOTOXIC IMPURITY IN NITROFURANTOIN BY GAS CHROMATOGRAPHY

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ABSTRACT
The purpose is to develop a method for identification and quantification of Ethyl 2-chloroacetate in Nitrofurantoin at trace level by using the simple gas chromatographic technique. The method was validated as per ICH guideline in terms of LOD, LOQ, Method precision, accuracy and specificity. The LOD and LOQ values were found as 0.38ppm and 1.1 ppm respectively.
Keywords: Development, validation, Ethyl 2-chloroacetate, Nitrofurantoin, Gas chromatography.

INTRODUCTION

Ethyl 2-chloroacetate is used as raw material in the manufacturing of Nitrofurantoin. Ethyl 2-chloroacetate is a clear colorless liquid with a fruity, pungent odor with a density of 1.145 g/mL and a boiling point of 143°C.It is a flammable compound having an empirical formula of $C_4H_7ClO_2$ and molecular weight of 122.55 g/mol

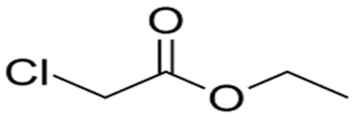

Fig-1: Ethyl 2-Chloroacetate

There are few methods available for the determination of Nitrofurantoin and its relative impurities in the literature by using HPLC, Stripping, Voltammetry and UV Spectrophotometry. The literature search clearly suggests that no analytical method is available for identification of Ethyl 2-chloroacetate in Nitrofurantoin as a genotoxic impurity. So important to develop a method for identification of Ethyl 2-chloroacetate in nitrofurantoin. Hence analytical method was developed for Ethyl 2-chloroacetate in Nitrofurantoin, which is a stability indicating method.

EXPERIMENTAL

Reagents and Chemicals

Ethyl 2-chloroacetate: Sigma Aldrich, LC grade n-hexane: Rankem.
Samples of Nitrofurantoin: Local market

Chromatography Conditions

Column	: Length 30 m and internal diameter 0.32 mm I.D x 1.0 µm DB-1
Detector	: FID
Inj temp	: 250 °C
Det temp	: 270 °C

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http://dx.doi.org/10.31788/RJC.2019.1211624

Column temp programme: Initial 60 °C with the hold of 5 minutes, raise rate of 40°C/min to final temperature 260 °C and hold for 20 minutes
Carrier gas : Helium
Flow : 1.0 mL/min (Constant velocity)
Mode : Split
Split ratio : 1:2
Injection volume : 2.0µL
Diluent : n-hexane (C_6H_{12})

Instrumentation
GC Agilent 6890B with autosampler
FID detector
Carrier Gas: Helium

Preparation of Standard Solutions
Ethyl 2-chloroacetate solution was prepared by weighing 37.5 mg of Ethyl 2-chloroacetate in a 100mL volumetric flask and make up to 100mL with diluent. Taken 2mL from the above flask and diluted to 50mL using diluent.
5mL above mentioned solution and diluted to 100mL. (Equivalent to 3.75ppm with respect to sample concentration 200mg/ml as per maximum daily dosage 400mg, i.e. TTC=1.5/daily dosage grams).

Test Solution
Weighed about 400 mg of Nitrofurantoin drug substance into 5 mL volumetric flask, added 2 mL of diluent (n-hexane) shake well and filtered through a 0.45 µ filter. The filtered solution used for sample injection.

RESULTS AND DISCUSSION

Method Development and Optimization
The purpose is to develop a method for identification and quantification of Ethyl 2-chloroacetate in Nitrofurantoin at trace level by using the simple gas chromatographic technique. Ethanol, acetone, diethyl ether and n-hexane(C_6H_{12}) were used as diluents for method development of Ethyl 2-chloroacetate.As Ethyl 2-chloroacetate shows good response compared to ethanol, acetone, and diethyl ether than C_6H_{12} Hence diluent was finalized as C_6H_{12}.Ethyl 2-chloroacetate is soluble in n-hexane and insoluble in water whereas Nitrofurantoin is soluble in dimethyl formamide and insoluble in C_6H_{12}. Hence the determination of Ethyl 2-chloroacetate in Nitrofurantoin was finalized with C_6H_{12} as diluent. The experiment was initially carried out on the DB-5(5% phenyl-95% dimethylpolysiloxane) column but replaced by DB-1 (100 % dimethylpolysiloxane) column for a sharp peak. The optimization was done for split ratio and injection volume up to 2 µL with a split ratio of 2:1 of sample concentration (200mg/mL).

Method Validation
The method validation was performed as per the ICH guidelines. The Validated method parameters include specificity, accuracy, sensitivity, precision, linearity, robustness, ruggedness and solution stability.

Table-1: Summary of method validation results for Ethyl 2-chloroacetate

Validation parameter	Acceptance criteria	The result (Ethyl 2-chloroacetate)
Specificity	The method should be specific	Specific
Precision		
System precision	% RSD not more than 15.0	1.61
Repeatability	% RSD not more than 15.0	1.13
Intermediate precision	% RSD not more than 15.0	1.40
LOD	The peak should be detected	0.38 ppm
LOQ	The peak should be quantified	1.1 ppm

	% RSD not more than 15.0	1.43
Linearity	Co-relation coefficient NLT 0.99	0.9989
Accuracy	Should be between 80-120%	98.2% to 100.4%
Robustness		
Flow (0.9 mL/min)	% RSD not more than 15.0	0.37
Flow (1.1 mL/min)	% RSD not more than 15.0	0.53
Column oven temperature (54°C)	% RSD not more than 15.0	1.16
Column oven temperature (66°C)	% RSD not more than 15.0	1.45

Table-2: Linearity Results

Level	Corrected Concentration (ppm)	Area Obtained
LOQ	1.13	3.71
50%	1.18	6.18
80%	3.00	9.89
100%	3.75	12.99
120%	4.50	14.83
150%	5.63	18.54
Slope		3.316
Y-intercept		0.039
correlation coefficient		0.9989

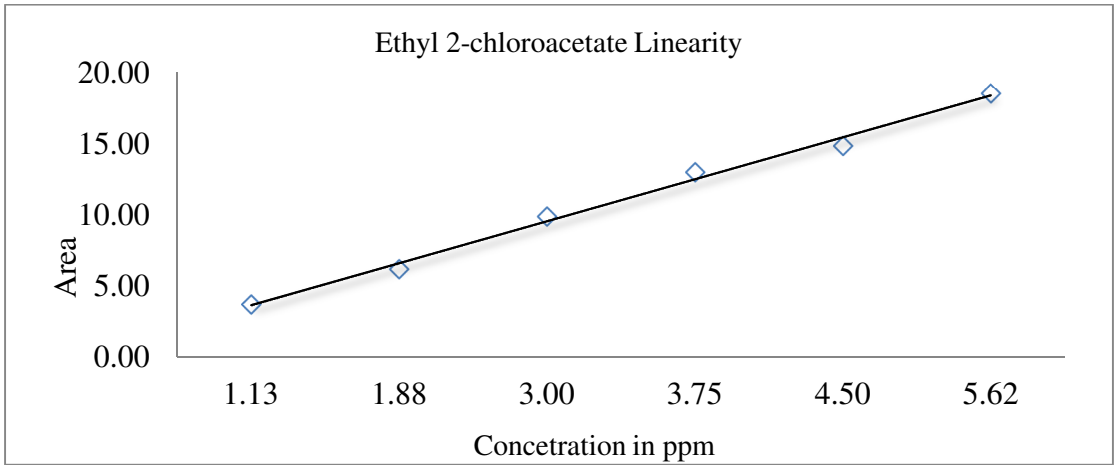

Fig.-2

Chromatograms

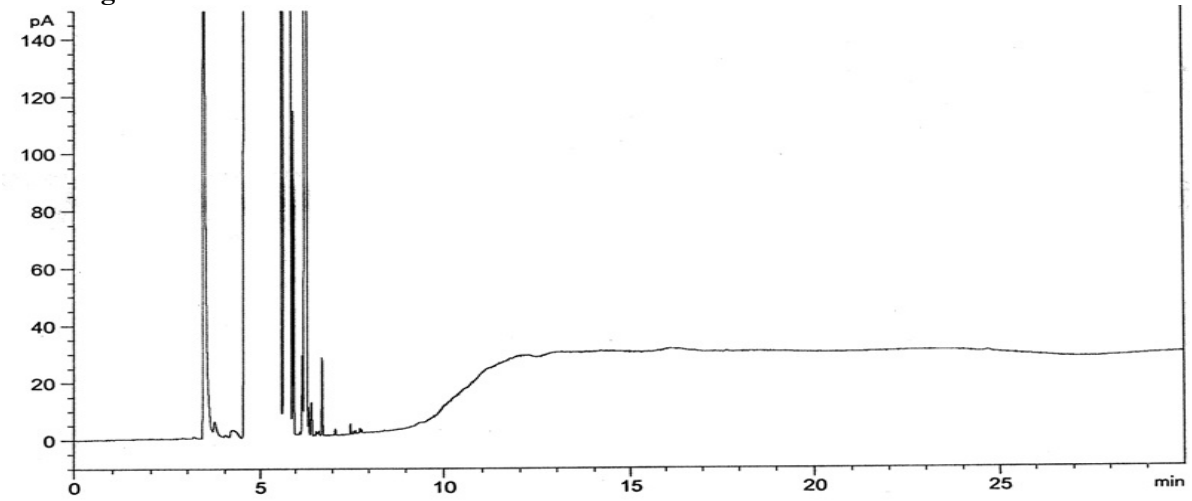

Fig-3: Blank Chromatogram

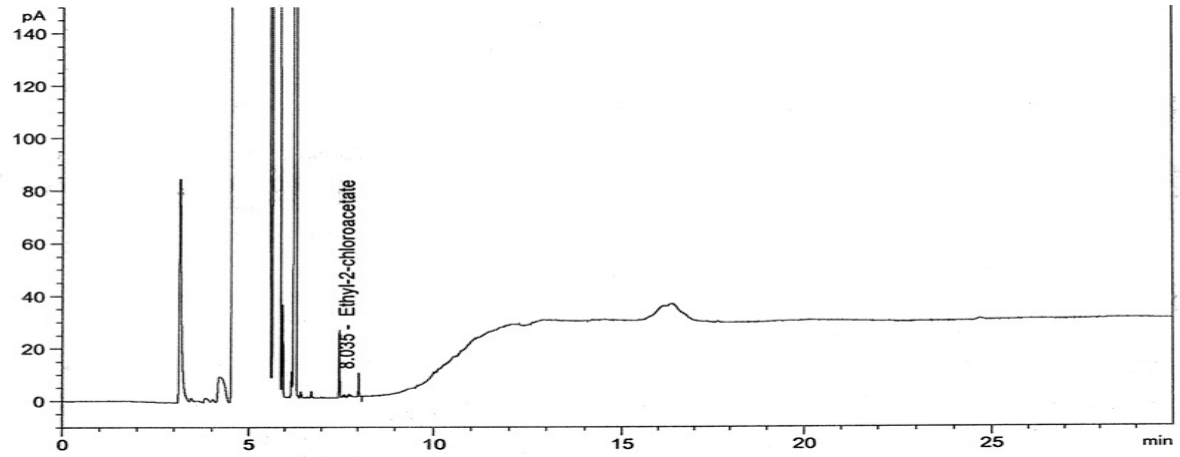

Fig.-4: Standard Chromatogram

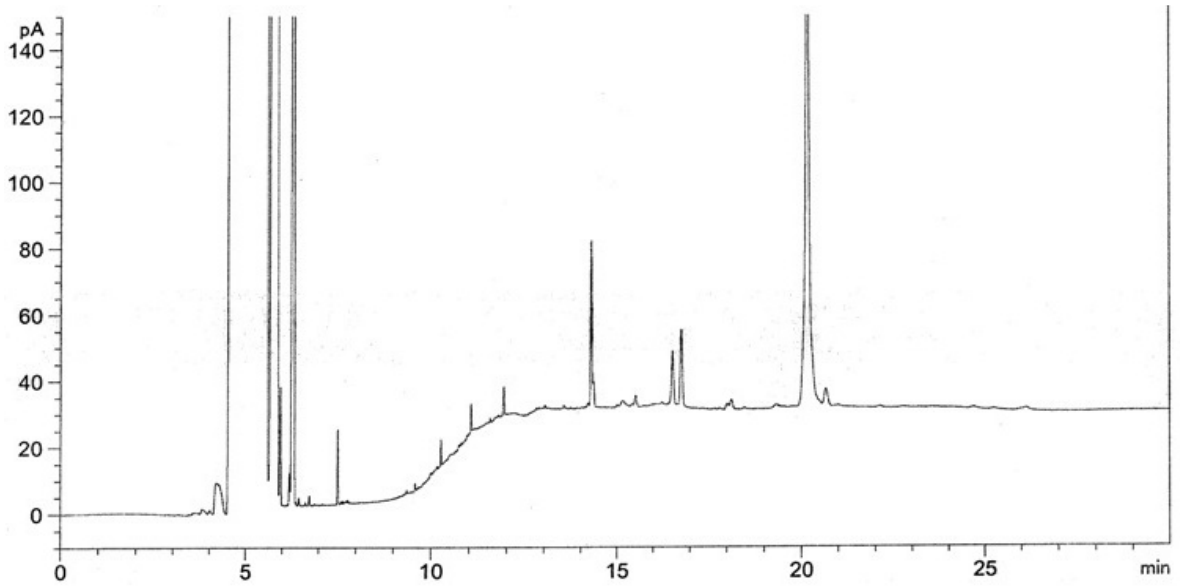

Fig.-5: Sample Chromatogram

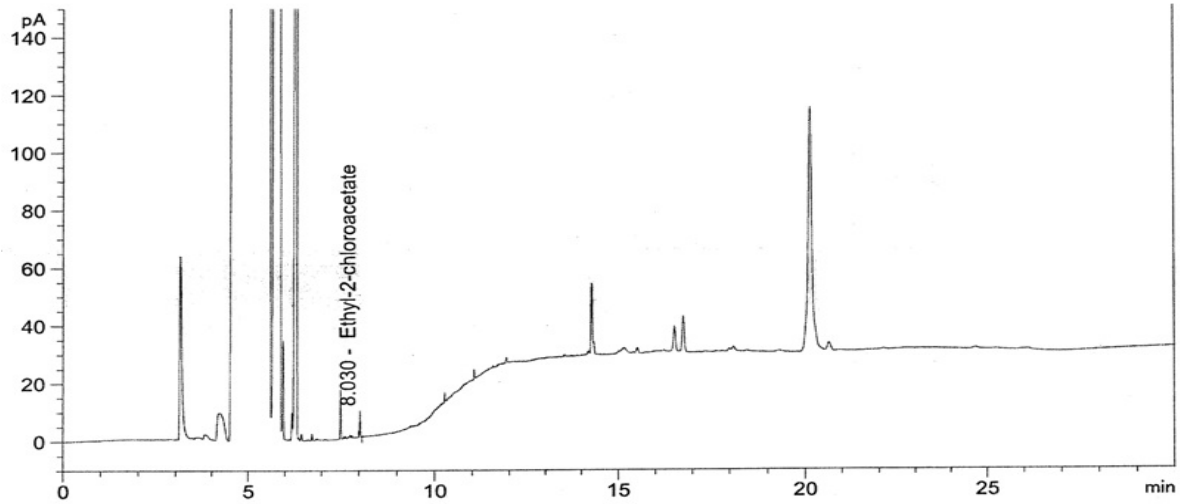

Fig.-6**:** Spiked Sample Chromatogram

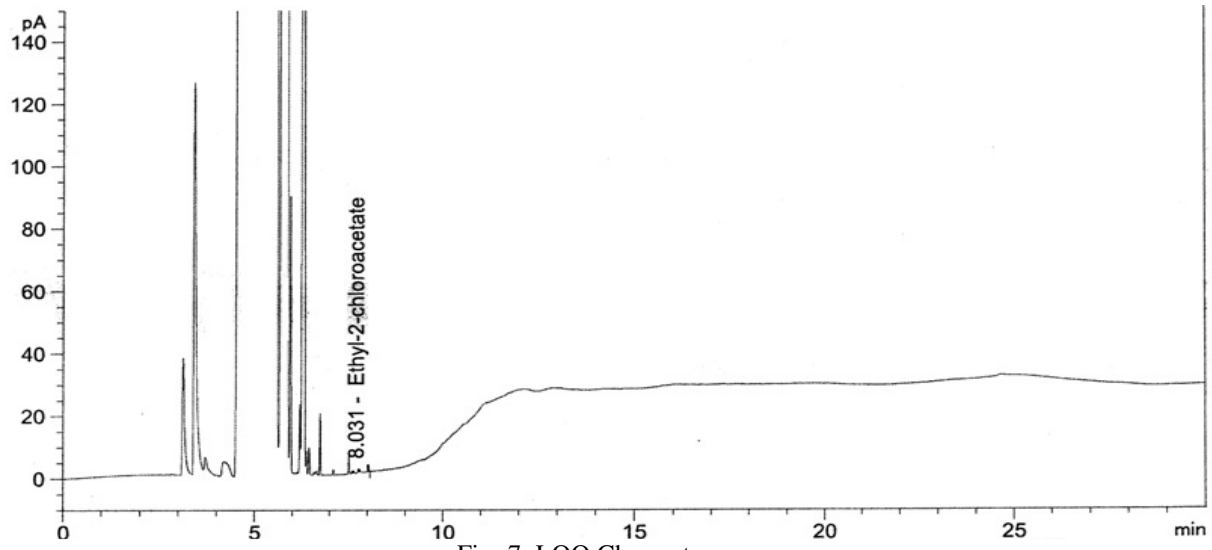

Fig.-7: LOQ Chromatogram

CONCLUSION

A simple and sensitive GC method has been developed and validated for the trace investigation of Ethyl 2-chloroacetate in pharmaceuticals. The validation has been conducted according to ICH guidelines. Compared with the previously reported methodologies, this method utilizes an FID detector, which is readily available in most of the testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive to detect 0.38 ppm and quantify1.1 ppm level of Ethyl 2-chloroacetate in drug substances.

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